

# Maximized photosynthetic capacity and decreased hydraulic failure risk during aging in the clump bamboo, *Bambusa chungii*

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## Highlights

- We depicted the anisohydric behavior of *Bambusa chungii* stomata to maximize carbon assimilation
- We found their structural compensation to maintain the hydraulic safety

## Abstract

To assess the water use of a clumped bamboo species, we investigated water use, physiological responses, and structural changes related to culm aging in the clumped bamboo species *Bambusa chungii*. Anisohydric behavior was characterized by the changed leaf water potential ( $\Psi_L$ ), constant stomatal conductance ( $g_s$ ), and the low stomatal sensitivity ( $-m$ ) in the young (0.52) and mature groups as well as the aged group (0.41). Intercellular  $CO_2$  ( $C_i$ ) was negatively related to  $g_s$ , especially during the dry season ( $R^2 = 0.62$ ). Hydraulic conductivity ( $k_s$ ) decreased by 57.9% and 58.8% in

the mature and aged groups. This was accompanied by a leaf area ( $A_L$ ) that decreased by 55.7% and 63.7% and water transport path ( $h$ ) that shortened by 8.5% and 23.3% to maintain the hydraulic safety. The photosynthetic rate ( $A_n$ ) was similar among the three age groups even during the dry season when water deficits occurred. This might be due to compensation by increased chlorophyll content (5.3% greater for the mature group) and stomata density (7.4% and 8.1% greater for the mature and aged groups). Physiological and structural regulation contributes to reproductive success for *B. chungii*.

**Key words:** Bamboo, TDP method, senescence, hydraulic balance, carbon assimilation.

## Introduction

Carbon and water cycle balances in forest ecosystems have changed profoundly by the direct or indirect effects of increased atmospheric temperature and altered precipitation patterns resulting from increased greenhouse gas levels (Schimel *et al.*, 2001; *et al.*, 2012). Knowledge of how physiological regulation and structural adaptations of plants are linked to environmental conditions provides the base for predicting their future development (Dudley, 1996). Bamboo plantations are widely planted in subtropical Asia, and they contribute to precipitation circulation, soil water conservation, and soil fertility improvement (Wang *et al.*, 2004; Christanty *et al.*, 1997; Nandy *et al.*, 2004), (Singh and Singh, 1999; Ohtsuka *et al.* 2007; Wang *et al.*, 2016).

Bamboo species are fast growing with well-developed rhizomes and clonal reproduction. They are able to rapidly colonize disturbed habitats and produce a bamboo-dominated forest canopy (Manges, 2000; Gagnon *et al.*, 2008; Wang *et al.*, 2016) that can significantly alter plant species composition compared to natural conditions (Zheng *et al.*, 2003). Bamboo has great potential for biomass production and might be a net sink for CO<sub>2</sub> carbon sequestration especially in native areas (Li *et al.*, 2000; Pearson *et al.*, 1994; Ohtsuka *et al.*, 2007). Leaf stomata simultaneously regulate both the influx of carbon dioxide and the efflux of water between a leaf and its environment (Anderson *et al.*, 2000), so substantial water loss would also be expected. The Moso bamboo forest has an annual transpiration of 567 mm,

accounting for ~32% of the annual precipitation (Komatsu *et al.*, 2010). This is quite similar to the 620.5-mm annual transpiration of the *Abies amabilis* forest (Martin *et al.*, 1997), and greater than the 250-mm annual transpiration of a *Fagus sylvatica* forest (Keitel *et al.*, 2003). However, shallow bamboo root systems are distributed mostly within the upper 0–20 mm of soil may not be well adapted for water uptake during drought conditions (Liese, 1987; Zhong *et al.*, 2014). In addition, the gradually decreased xylem conductivity in secondary growth also increases the possibility of hydraulic failure (Liese and Weiner, 1996; Kleinhenz and Midmore, 2001). Hydraulic failure occurs when the water in xylem conduits cavitates under high tension. The resulting embolisms restrict water transport to the canopy, ultimately resulting in tissue death due to desiccation. Clarification of the relationship between large transpiration demand and a vulnerable conductive system increases our understanding of plant responses to variable resource supplies (Onozawa *et al.*, 2009; Dierick *et al.*, 2010). Few studies have addressed how bamboos maximize carbon assimilation while maintaining conductive system hydraulic safety.

Generally, stomata respond to increasing vapor pressure deficit between leaf and air by partial closure (Oren *et al.*, 2001), avoiding high transpiration and a water potential decline (Liese and Weiner, 1996). This prevents excessive dehydration and physiological damage to the photosynthetic apparatus but decreases carbon assimilation (Manzoni *et al.*, 2013). However, some species have low midday leaf water potential as soil water potential decreases with drought while maintaining high stomatal conductance with increasing VPD — an example of anisohydric behavior (West *et al.*, 2008). It is not known if such behavior exists in bamboo species during growth when they balance water consumption needs with hydraulic limitations in response to environment conditions. Some anisohydric shrubs and herbs wilt in response to extreme drought stress to avoid sustained damage to their photosynthesis functions (Chiariello *et al.*, 1987; Tardieu *et al.*, 1996, Fortini *et al.*, 2003; Schultz, 2003). Many studies have reported isohydric (reduced stomatal conductance to limit transpiration) behavior in bamboo. Some adaptations to water stress such as leaf rolling by unique bulliform cells have also been found in Gramineae species (including bamboo) (Zheng *et al.*, 2001). In this manner, the leaf area as well as the boundary layer conductance are reduced (Dauzat *et al.*, 2001; Kadioglu *et al.*, 2011). We hypothesize that anisohydric behavior might also be a water stress regulation strategy

for bamboo.

Age-related tree structure can significantly influence plant water regulation. Some researchers propose that plant structure is prone to converge on adaptations most suitable to a given set of conditions (Meinzer *et al.*, 2004; McJannet *et al.*, 2007). However, this conclusion has been validated only for dicotyledon species. These species have active cambium for the production of new xylem tissues to maintain the ability to transport water and to avoid hydraulic limitations caused by aging and allometric growth. In contrast, water use by monocotyledon species such as bamboo may not exhibit allometric change with age because secondary growth is absent. The hydraulic system of bamboo, formed during the early growth stages, will not change with age. However, increased embolization by methylcellulose can block conduits within the xylem, leading to a decline of hydraulic conductivity (Liese and Weiner, 1996). The physical limitations on water flow through hydro-active tissue (sapwood in the xylem) influences stomatal behavior and transpiration in trees, which can be expressed, based on Darcy's law, as

$$J_s = k_s \frac{\Delta \Psi}{h} \quad (1)$$

where  $J_s$  is the sap flux density,  $k_s$  is the sap wood specific hydraulic conductivity (whole-plant conductance per unit sapwood).  $\Delta \Psi$  represents the water potential difference between rhizosphere and leaf, and  $h$  is the tree height. This equation can be used to quantify the change in whole tree water conductivity. If isohydric behavior is present in bamboo water use regulation, based on this equation, the decreased  $k_s$  caused by physical limitations will be compensated by the reduced leaf water status ( $\Delta \Psi$ ) if  $J_s$  is related carbon assimilation and  $h$  is assumed to be constant, which will put the bamboo species in danger of hydraulic failure. Thus, other hydraulic (architectural or physiological) regulations are expected. However, it remains unclear if bamboo species can maintain anisohydric behavior and still optimize carbon assimilation as the plants age.

Among the Asia-Pacific region countries, China has the highest bamboo diversity (626 species) (Bystriakova *et al.*, 2003). The greatest bamboo species richness occurs in the forests of south China. Morphologically, all bamboo species can be categorized

as either monopodial or sympodial (Banik, 2015; Valade and Dahlan, 1991). Monopodial bamboos are native to temperate climates with cool wet winters, while sympodial bamboos are adapted to tropical climates with a pronounced dry season (Kleinhenz and Midmore, 2001). The tight clumping habit of tropical species exposes less rhizome surface to dehydration during extended dry seasons (Farrelly, 1984). In this study, we attempt to characterize the hydraulic regulation, at different ages, of a sympodial species, *Bambusa chungii*. Specifically, we determine if anisohydric behavior for the stomatal control of water use exists in this species and if it is maintained with aging. We test hydraulic compensation for the decreased xylem water conductance of the *B. chungii* culm and determine if carbon assimilation is maintained.

## Materials and methods

### 1. Experimental site and sap flow measurements

The field observations were conducted from January to December 2014 in the South China Botanical Garden in Guangzhou (23°10'39.9"N, 113°21'17.6"E). This is a transitional zone between tropical and subtropical regions. The entire year is divided, by hydrothermal conditions, into a wet season that extends from April to September and a dry season that extends from October to the following March (Zhu *et al.*, 2013). The altitude of this site is above 41 m, with an annual precipitation of 1,612–1,909 mm and a mean annual temperature of 21.4–21.9°C. The minimum and maximum temperatures usually occur in February and July, respectively.

Three clumps of *B. chungii* with an average density of 180 individuals were chosen for sap flow measurements. Fifteen individuals in each clump were chosen and classified by age (1–2 years, 3–4 years,  $\geq 5$  years, corresponding to young, mature and aged individuals, respectively) according to the morphological differences that characterize different growth stages (Zhao *et al.*, 2016). Sap flux densities were calculated using the equation of Granier (1987) with newly derived parameters in the unpublished paper (unpublished data), where we shortened the original 20 mm TDP probes to 5 mm and did the calibration both with a cut experiment of a culm segment under laboratory conditions and a pot experiment under field condition. Detailed mounting procedures of *in situ* measurements refer to measurements made on woody

species (Granier, 1987; Schäfer *et al.*, 2000) except that the two probes were installed across the bamboo knots, namely on both sides of a knot.

## 2. Stand parameters

Diameter at breast height (DBH) of all sample bamboo culms was determined with a diameter tape. The cross-section area ( $A_C$ ) of the culm wall was determined by placing a hook to cover the wall depth through a hole of 2 mm in diameter drilled into the wall (Figure S1). The depth covered by the hook corresponded to the wall thickness. The depth of four orientations was measured and averaged to improve  $A_C$  accuracy. After the field measurements were taken, twelve individuals (4 for each age group) were cut down to collect all of the leaves and measure the height (H) and numbers of knots ( $n_k$ ). Subsamples of leaves (5) for each individual were used to measure the leaf area with a portable leaf area meter (Licor-3000, USA) and the total fresh weight with an electric balance. The total leaf area ( $A_L$ ) of a culm was estimated by the relationship between fresh mass and leaf area derived from the subsamples collected on each individual. We fitted  $A_L$  to DBH to estimate the  $A_L$  of sample culms for sap flow measurements. H was unrelated to DBH, but it was positively related ( $R^2 = 0.68$ ) with  $n_k$ , which was expressed as

$$(2)$$

## 3. Canopy stomatal conductance

In forests where transpiration is well-coupled with atmosphere conditions, the mean stomatal conductance can be calculated based on a simplified equation (Köstner *et al.* 1992). Assuming that the  $J_s$  are scaled by the ratio of cross-section area of culm wall and leaf area ( $A_C/A_L$ ) is equal to the transpiration rate per unit of leaf area ( $E_L$ ). The mean stomatal conductance for individual culms,  $G_s$ , can be calculated as:

$$(3)$$

where  $E_L$  is whole-culm transpiration per unit leaf area ( $\text{g m}^{-2} \text{s}^{-1}$ ),  $G_v$  is the universal gas constant adjusted for water vapor ( $0.462 \text{ m}^3 \text{ kPa}^{-1} \text{ kg}^{-1}$ ),  $T_a$  is the air temperature (K),  $\rho$  is the density of water ( $998 \text{ kg m}^{-3}$ ), and VPD is in kPa.  $G_s$  is in units of  $\text{mmol m}^{-2} \text{s}^{-1}$  (Monteith & Unsworth, 2013). In this study, the bamboo canopy was open and

met the application requirements of equation (3).

Leaf area ( $A_L$ ) was assumed to be equal in dry and wet seasons, and the  $G_s$  calculation is therefore not subject to the seasonality of leaf area dynamics. It was estimated after (1) performing a cross-correlation analysis between VPD and  $F_d$ , and using the time lag to infer a time-corrected  $F_d$  and (2) filtering out data for VPD < 0.6 kPa in the hours of early morning and late afternoon (Oren *et al.* 1999).

#### 4. Stomatal sensitivity to vapor pressure deficit

Granier *et al.* (1996) proposed that stomatal sensitivity is proportional to the magnitude of  $G_s$  at low VPD (VPD = 1 kPa) when soil moisture is not limiting, which was expressed as:

$$(4)$$

where  $G_{Sref}$  is the intercept (i.e., the value of  $G_s$  at VPD = 1 kPa in a log-linear relationship), and  $-m$  is the slope of the regression fit representing stomatal sensitivity to VPD (i.e.,  $dG_s/d\ln VPD$ ). Oren *et al.* (1999) demonstrated that  $m$  is approximately 0.6.

In this study, the  $-m$  and  $G_{Sref}$  in the wet and dry seasons were used to delineate the stomatal control of transpiration. Thus, a boundary line analysis of the relationship between VPD and  $G_s$  was performed following the principle proposed by Schäfer *et al.* (2000) after excluding nighttime data ( $Q_0 = 0$ ). The relationship between  $\ln VPD$  and  $G_s$  of each small subset was linearly fitted, and the intercept and slope corresponded to the  $G_{Sref}$  ( $G_s$  at VPD = 1 kPa) and the sensitivity in response to VPD ( $dG_s/d\ln VPD$ ,  $\text{mmol m}^{-2} \text{s}^{-1} \text{kPa}^{-1}$ ), respectively (Oren *et al.* 1999). Then, the relationship between  $G_{Sref}$  and  $-d\ln d/dG_s$  for both seasons was linearly fitted to estimate  $-m$ .

#### 5. Leaf gas exchange and physiological characters

Daily courses of leaf net photosynthetic rate ( $A_n$ ), stomatal conductance ( $g_s$ ), transpiration rate ( $E_t$ ) were measured at 2 h intervals from 5:00 am to 7:00 pm with a portable photosynthesis measurement system (LI-6400, Li-Cor, Lincoln, NE, USA) under ambient conditions on typical clear days during the dry (January 15–17, 2014)

and wet seasons (July 19–21, 2014). Ambient CO<sub>2</sub> concentration was ~390 mmol mol<sup>-1</sup>, air humidity ranged from 55–65%, leaf temperature was between 12°C and 28°C and VPD between 1.2 and 1.5 kPa. For each measurement, leaves were exposed to the above conditions for 2–5 min to allow the stabilization of photosynthetic parameters. Three sun-exposed mature shoots from each culm of each age group were selected for the measurement. Leaf water potential ( $\Psi_L$ ) was measured with a pressure chamber (PMS, Albany, OR, USA) on the detached shoots after the gas exchange measurements. Chlorophyll content and stomatal density were determined in four leaves collected from the culms selected for gas exchange measurement. For the chlorophyll content determination, the fresh leaves (0.1–0.2 g) were cut into strips of ~1 mm in width and soaked in the mixture of acetone and ethyl alcohol (1:2, 30 ml) for 24 h. The extract solution was used to measure chlorophyll content with a spectrophotometric method that was calculated according to Arnon's equation (Arnon, 1949). The number of stoma was counted in a fixed field of vision under an optical microscope to calculate the leaf stomatal density. The diameter of stoma was regarded as a proxy for the stomatal size.

## 6. Statistical analysis

ANOVA was used to compare differences in parameter values in different age groups and seasons. Statistical analyses were performed with SAS 9.2 (Statistical analysis system, NC, USA). For graphing, Origin 9.0 (Origin Lab, Northampton, MA, USA) was used.

## Results

### 1. Sap flow response to PAR and VPD

On a daily basis, PAR and VPD induced leaf transpiration changes and leaf water status, which drives sap movements (Green *et al.*, 2003). Sap flow increased in the morning with increasing daylight intensity, reached a maximum around 12:00 p.m. noon, and then gradually decreased until PAR = 0, in both dry and wet season (Figure 1). Sap flow data for all the culms in the wet season was used to quantify the indirect regulation of PAR and VPD with Partial Correlation Analysis. This showed a PAR-dominating sap flow variances ( $0.63 < r < 0.96$  with a mean of 0.88,  $p < 0.001$ ), but



no dominance of VPD (mean  $r = 0.13$ ,  $p > 0.05$ ). We continually fitted the exponential relationship between PAR and  $J_s$  for each culm ( $0.68 < R^2 < 0.89$ ) to obtain the predicted  $J_s$  ( $J_{s,p}$ ) from the fitted curves. The  $J_{s,p}$  of each culm was then normalized by their maximum (at max PAR) and averaged by all the culms within each group. Figure 2a shows the response of normalized  $J_s$  ( $J_{s-N}$ ) to PAR. Different response sensitivity to PAR was found among the three age groups. The PAR at  $J_{s-N} = 0.8$  was 557.7, 1074.3 and 856  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for young, mature and aged groups. We chose the  $J_s$  during the time period of 11:00 – 15:00 to fit with VPD and found a constant transpiration in a VPD range of 1.7–3.0 kPa for all the tree age groups (Figure 2b). Accordingly, the mean  $J_s$  was 21.9, 13.4 and 17.4  $\text{g m}^{-2} \text{s}^{-1}$  with significant differences among all the age groups ( $p < 0.01$ , ANOVA).

### 1.1 Leaf physiological characters

$A_n$  reached a maximum of 6.9  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , 4.7  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and 5.2  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for young, mature and aged individuals before the “midday depression” under high radiation ( $\text{PAR} > 910.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for all groups in wet season; however, this depression was not observed in the dry season (Figure 3a).  $A_n$  differences among groups were not significant except at 9:00 ( $p = 0.02$ ) in the wet season. The daily mean of  $A_n$  was much higher in the wet season ( $3.4 \pm 1.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) than in the dry season ( $1.8 \pm 0.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) ( $p < 0.001$ ).  $g_s$  showed daily variation similar to  $A_n$ , while no significant difference was found between dry and wet season ( $0.018 \pm 0.005 \text{ mol m}^{-2} \text{s}^{-1}$  and  $0.02 \pm 0.01 \text{ mol m}^{-2} \text{s}^{-1}$  respectively,  $p = 0.49$ ).  $E_t$  values for young and aged individuals were significantly higher than for mature ones (Figure 3c,  $p = 0.036$ ) in the wet seasons, especially when light intensity was high, which was consistent with  $J_s$  (Figure 1c, d). However, no differences were observed among the age groups in the dry season. Leaf water potential ( $\Psi_L$ ) decreased rapidly after 7:00 and recovered after 11:00 when the stomata partially closed at 9:00 (Figure 3d). A rapid increase of transpiration occurred after 13:00 (Figure 3c) when the stomata reopened. This pattern mirrored the dramatic consumption of water from the stem in the morning (before 11:00 am). In addition, we found a  $\Psi_L$  sequence of young  $>$  aged  $>$  mature that was significant in the dry season ( $p < 0.05$ ), and positively related to their  $J_s$  and transpiration (Figure 2b).

In addition, we also found a seasonal difference in the dynamics of intercellular CO<sub>2</sub> (Ci) (Figure 4a). Ci started to decrease gradually at 6:00 in response to the increase of An with the rising PAR until 9:00, and increased again after 15:00, in the dry season. We did not conduct measurements at 5:00 in the wet season and it was predicted that Ci had the same value as in the dry season before sunrise (6:00). We observed that Ci decreased to a very low value (mean of 70.5  $\mu\text{mol CO}_2 \text{ mol}^{-1}$  for all culms) at 7:00 in the morning, which was caused by the rapidly increasing PAR (Figure 4a) before being promoted by increased  $g_s$  at 9:00, and then decreasing again at 11:00 until 17:00 when it recovered. No significant differences were observed among the age groups. It seems that Ci has a dynamic trade off with  $g_s$ . Thus we fitted Ci to  $g_s$  for all the individuals and found that they were negatively related, especially during the dry season (Figure 4b).

## 1.2 Stomatal response to Ci

We fit the relationship between  $G_{\text{Sref}}$  and  $-\text{dlnVPD}/\text{d}G_s$  of the three age groups for both dry and wet seasons (Figure 5). The young group had the highest slope (i.e.,  $-m$ ) of 0.52 ( $p < 0.01$ ) without differences between the two seasons. The slopes of the mature and aged group were similar ( $p > 0.05$ ). They had slopes of 0.40 and 0.41 in the wet and the dry season; the  $-\text{dlnVPD}/\text{d}G_s$  in the wet season tended to be higher than in dry season. All the  $-m$  values were significantly lower than the standard value of 0.6 proposed by Oren *et al.*(1999). This indicated that the stomas were partially decoupled from the VPD induced stomatal regulation (decreased  $g_s$ ) when VPD was very high.

## 1.3 Age effects on culm form features and leaf morphology

At the whole-culm level, both H and  $A_L$  significantly decreased with age (Figure 6a, b). Meanwhile,  $A_C$  unexpectedly decreased (by 13.1% and 8.1%) for mature and aged (data not shown). The climatic variations among the adjacent years induced growth differences of bamboo shoots might be responsible for such changes among the age groups. We found a gradual increase (decrease) in annual mean relative humidity (temperature) from 2009 to 2014 (corresponding to the aged to young age groups), which was favorable for the growth of younger bamboo (Zheng *et al.*, 2001). We estimated the individual height of nine culms that were used to conduct  $\Psi_L$

measurement based on equation 3 and calculated their  $k_s$  combining  $\Psi_L$  and  $J_s$  in the dry season with equation 1. The  $k_s$  of the mature and aged individuals decreased by 57.9% and 58.8% compared to those of the young groups, which revealed that conduits were significantly blocked as they grew older. At the leaf level, mature groups had the highest chlorophyll content (Chl) of  $1.02 \text{ mg g}^{-1}$ , while levels of the young and aged groups were  $0.97 \text{ mg g}^{-1}$  and  $0.75 \text{ mg g}^{-1}$ , respectively (Figure 6c). In contrast, young groups had the lowest leaf stomatal density than the other two age groups. Stomatal density in the young group was 6.9% and 7.5% lower than the mature and aged groups, respectively (Figure 6d).

## Discussion

Stomatal control in woody species under high light conditions is an adaptation for maintaining leaf water potential in situations of excessive transpiration (Oren *et al.*, 2001; Nilson and Assmann, 2007). We found a rapid decrease of  $g_s$  that began at 9:00 when  $\text{PAR} = 910.7 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$  with a mean  $\Psi_L$  of  $-1.25 \text{ MPa}$  in the wet season. However, this did not occur in the dry season with a significantly lower mean  $\Psi_L$  ( $-2.53 \text{ MPa}$ ) when PAR reached a maximum of  $792.8 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$  (Figure 1a). In addition, we compared  $g_s$  of all the culms at 11:00 (Figure 3b), and found no differences among age groups or between seasons ( $p > 0.05$ ). This implied that the stomatal control might not be a complete response to leaf water status for bamboo. The constant  $J_s$  with increased VPD (Figure 2b) also mirrored weak stomatal control of transpiration in the dry season as  $\Psi_L$  was quite low (Figure 3d).

However, the decreased  $g_s$ , shown in Figure 4, indicated a  $C_i$  induced stomatal regulation, especially during the dry season. The regulation of stomatal conductance is a response to release of leaf abscisic acid (ABA) that tends to be activated when pH is low (for example when  $C_i$  is high) (Ackerson *et al.*, 1982; Kaiser *et al.*, 1981), which eventually leads to stomatal closure. Thus, the rapidly increased  $C_i$  in our study between 9:00–11:00 in the wet season could be responsible for the stomatal closure at 9:00 and for the PAR-dominating  $J_s$  (Figure 2a). This has already been demonstrated in the bamboo *Indocalamus barbatus* that had a more profound “midday depression” (decreased  $g_s$ ) when exposed to simulated acid deposition (Xie *et al.*, 2008). The traditional view of plant leaf water potential induced stomatal control (Nilson and

Assmann, 2007) may not be true for *B. chungii*. ABA induced stomatal regulation is an acclimatory response to drought in some herbs, shrubs and tree species (Chaves *et al.*, 1987; Socias *et al.*, 1997). As a result, increased water use efficiency occurs under moderate stress in these plants due to the maintenance of photosynthesis (Socias *et al.*, 1997). These results imply a reduced effect on photosynthesis caused by the leaf water status and the maintenance of high carbon assimilation in bamboo even under desiccating conditions (high VPD).

Our results indicate that *B. chungii* may not maintain the minimum leaf water potential during the dry season. It maintained  $g_s$  when the leaf water potential was low, showing anisohydric behavior. The relation of stomatal conductance and VPD showed that  $-m$ , in all three age groups, was significantly less than the standard value of 0.6 ( $p < 0.01$ ), especially for the mature and aged groups (mean = 0.41). Oren *et al.* (1999) reported that if stomata do not regulate the leaf potential near a constant value (anisohydric behavior), a lower slope is expected. Anisohydric behavior has been observed in many plant groups including species such as juniper (*Juniperus formosana*), sugar maple (*Acer saccharum*), sunflower (*Helianthus annuus*), and eucalyptus (*Eucalyptus gomphocephala*). Anisohydric behavior allows a greater  $\Psi_L$  range than that which occurs in isohydric species (West *et al.*, 2008; Franks *et al.*, 2007). Anisohydric species benefit from higher rates of gas exchange during drought, however, at the risk of a higher probability of hydraulic failure (West *et al.*, 2008). These plants are more likely die of cavitation rather than carbon starvation (McDowell *et al.*, 2008). As illustrated in Figure 3a and b, the significant reduction of leaf water potential during the dry season did not lead to a decrease of  $g_s$  and  $A_n$  at noon, and no differences of  $g_s$  were found between wet and dry seasons. The  $\Psi_L$  reached a minimum of  $-3.1$  MPa for the mature group in dry season. The wilting point leaf water potential ( $\pi_{tp}$ ) across 71 tree species in tropical forest ranged from  $-1.4$  MPa– $-3.1$  MPa, and only 1.5% of the species had  $\pi_{tp}$  lower than  $-3.0$  MPa (Maréchaux *et al.*, 2015). Despite the maintenance of  $g_s$  in dry season,  $A_n$  still suffered a significant decrease, which may be an indication of the damage to the photosynthetic apparatus induced by drought stress (Foyer *et al.*, 1994; Wingler *et al.*, 2000). Further studies on photosynthetic characteristics, such as chlorophyll fluorescence, are needed to clarify these results.

Our results also showed a non-significant difference in  $A_n$  among the three age groups in both seasons (Figure 3a) and when leaves suffered a significant water deficit during the dry season (Figure 3d). Meanwhile, the mature and aged groups tended to be more anisohydric than the young group with a lower stomatal sensitivity ( $-m = 0.40$  and  $0.41$ ), but with a greater negative  $\Psi_L$  value in both seasons (Figure 5). The biosynthesis of ABA can be triggered by a reduction in leaf turgor in angiosperms — a higher sensitivity of ABA synthesis to leaf turgor corresponded with a higher stomatal sensitivity (higher  $-m$ ) to VPD (McAdam and Brodribb, 2016). Thus, decreasing  $-m$  with increased plant age may relate to the partial loss of enzyme activity for the ABA synthesis. This possibility needs further verification. Our results suggest a stronger tendency to maximize carbon assimilation in the mature and aged groups. This is in consistent with the study results of Emanuel *et al.* (2007). The carbon-sequestering ability of five-year-old bamboo (*Phyllostachys pubescens*) was significantly higher than three- and one-year-old bamboos during the peak growth period (Zhong *et al.*, 2014). The increased chlorophyll content (Figure 6c) for mature groups and higher stomatal density for mature and aged group (Figure 6d) might be associated with this tendency (Xu and Zhou, 2008). Mature and aged bamboos transfer ~80% of all the nutrient storage to the bamboo shoots, which significantly contributes to their reproductive and dispersal success (Kleinhenz and Midmore, 2001).

However, weak stomatal control would expose the bamboo to the risk of hydraulic failure since the shallow roots and decreased conductivity cannot provide sustained available water for transpiration. The water content in bamboo stems decreased significantly during a maturation phase of up to three years (Sattar *et al.*, 1994). In *B. chungii*, a significantly decreased  $J_s$  and its sensitivity to PAR were seen in the mature and aged groups (Figure 2). Thus, a decrease in stomatal conductance as well as in transpiration rate was not unexpected as this has been found previously in woody tree species (Delzon and Loustau, 2005). However, such a change in  $E_t$  and  $g_s$  was not observed for *B. chungii* bamboo, especially under high light conditions (Figure 1b, c). Instead, the young group had the minimum  $E_t$  and  $g_s$  with a mean of  $0.77 \text{ mmol m}^{-2} \text{ s}^{-1}$  and  $0.0187 \text{ mol m}^{-2} \text{ s}^{-1}$  during the wet season. The hydraulic architectures are reported to be convergent in plant water regulation (Zeppel *et al.*, 2013). Whitehead *et al.* (1984) proposed an identity equation showing the manner in

which  $g_s$  and  $\Psi_1$  are linked to structural features such as the ratio of leaf area to sapwood area ( $A_L/A_C$ ), axial flow path length ( $h$ ), and xylem permeability ( $k_s$ ):

$$(4)$$

where  $\Psi_s$  is the soil water potential,  $g$  is gravitational acceleration,  $h$  is the vertical distance, and  $\rho$ ,  $\eta$  and  $\rho_w$  are the density, dynamic viscosity and molar volume of water, respectively. To maintain hydraulic balance for an given  $g_s$ , the decreased  $k_s$  (lower by 57.9% and 58.8% in mature and aged groups) in bamboo must be offset either by a reduction in  $h$  and  $A_L/A_C$  or in  $\Psi_1$ . Culm height is expected to be constant after one year of growth (Liese, 1987), but a decrease of 8.5% and 23.3% in culm height for mature and aged groups, compared to that of young culm height, was found in this study. The decreased  $h$  is associated with dead tissues at the top of the stem due to hydraulic failure associated with the growth sequence of bamboo shoots (up to down, Zhong *et al.*, 2014).  $A_L$  also declined by 55.7% and 63.7% (Figure 6a). A significant decline of  $A_L$  also occurs in the bamboo species, *Phyllostachys heterocycle*, as they age (Zhao *et al.*, 2016). Thus, the compensation for decreased  $A_L$  and  $h$  allows for a decreased  $\Psi_1$  that in turn maintains hydraulic safety. These results suggest an adaptive adjustment of the hydraulic architecture to cope with the conduits that are blocked by tyloses in the stems (Liese, 1987). Although the decrease of individual carbon assimilation caused by decreased  $A_L$  may not be completely compensated, the increased life span eventually contributes more to the carbon sink for each individual.

## Conclusion

*B. chungii* is a fast-growing species with significant eco-hydraulic effects. It maximized carbon assimilation by anisohydric behavior from the beginning of growth, while xylem cavitation and embolization triggered structural changes such as decreased leaf area and water path length possibly by reducing the tissues required to maintain hydraulic safety. To compensate for the reduced carbon assimilation caused by decreased  $A_L$ , the mature and aged groups improved their photosynthetic capacity by way of increased stomata density, higher chlorophyll content, and enhanced anisohydric behavior in response to water deficit. This helped older plants to maintain

an  $A_n$  similar to the young groups as well as to maximize their fitness.

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## Figure Legends

**Figure 1.** Daily course of PAR, VPD (a, b) and sap flux density ( $J_s$ , c, d) in dry and wet seasons.

**Figure 2.** (a) Normalized sap flow density ( $J_{s-N}$ ) of each age group (by their maximum) plotted against photosynthetically active radiation (PAR) and (b) the sap flow density ( $J_s$ ) in the time period of 11:00–13:00 in response to VPD in dry season (2014.01.01-2014.01.10). Bars indicate the  $\pm$  standard error of all the culms within the age group.

**Figure 3.** Daily dynamics of net photosynthetic rate ( $A_n$ ), stomatal conductance ( $g_s$ ), leaf level transpiration ( $E_t$ ) and leaf water potential ( $\Psi_L$ ) of three *B. chungii* individuals in each age group for three clear days in dry (January 16–18, 2014, open symbols and dash lines) and wet seasons (July 19–21, 2014, solid symbols and lines). For b, c, d, e and f, different symbols stand for the young (square), mature (circle) and aged (triangle) groups. Error bars correspond to the  $\pm$  standard error of three individuals in each group.

**Figure 4.** (a) Daily dynamics of intercellular CO<sub>2</sub> (Ci) in dry (January 16–18, 2014, open symbols and dash lines) and wet seasons (July 19–21, 2014, solid symbols and lines) and (b) Ci in relationship with leaf stomatal conductance (g<sub>s</sub>). Bars refers to the standard error of the culms within each age group (n = 15), the dash and lines are the least squares fit in dry and wet season, the asterisk is the predicted data of Ci in wet season at 5:00 am.



**Figure 5.** Sensitivity of mean stomatal conductance ( $G_s$ ) of bamboo culms of each age group in response to increasing vapor pressure deficit ( $-dG_s/d\ln D$ ) as a function of canopy stomatal conductance at  $D = 1$  kPa ( $G_{sref}$ ) in dry and wet seasons.

**Figure 6.** (a) Leaf area ( $A_L$ ), conductive area ( $A_C$ ) and (b) tree height (H), (c) chlorophyll content (Chl) and (d) stomatal density of *B. chungii*, for each age group. Vertical bars are one standard deviation. Different letters refer to significant differences at the 0.05 level.